Contractor's Report to the Board

Evaluation of Health Effects of Recycled Waste Tires in Playground and Track Products

(Publication #622-06-013)

Produced under contract by:

OEHHA
Office of Environmental Health Hazard Assessment

January 2007

Appendix C:
Raw Data From
Skin Sensitization Testing

INTEGRATED
WASTE
MANAGEMENT
BOARD

Product Safety Laboratories
2394 Highway 130
Dayton, NJ 08810
Tel: 732.438.5100
Fax: 732.355.3275
e-mail: PSL@productsafetylabs.com

PRODUCT

Rubber Tiles, EPDM Tiles, High Density Polyethylene, and Rubber Crumb

STUDY TITLE

Delayed Contact Dermal Sensitization Test - Modified Buehler Method for Solid Materials

DATA REQUIREMENT

U.S. EPA Health Effects Test Guidelines, OPPTS 870.2600 (2003)

AUTHOR

Daniel J. Merkel, B.S.

STUDY COMPLETED ON

PERFORMING LABORATORY

Product Safety Laboratories

2394 Highway 130

Dayton, New Jersey 08810

LABORATORY STUDY NUMBER

17608

PSL PRODUCT SAFETY LABORATORIES

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA 10 (d) (1) (A), (B) or (C).

Company: **CALIFORNIA EPA,**
OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

Company Agent:

_____	_____
Name	Title
_____	_____
Signature	Date

PRODUCT SAFETY LABORATORIES

GOOD LABORATORY PRACTICE STATEMENT

Rubber Tiles, EPDM Tiles, High Density Polyethylene, and Rubber Crumb

This study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA) with the following exceptions:

1. Specific information related to the determination of the stability, identity, strength, purity, and composition of the test substance as received and the concentration as tested is the responsibility of the study Sponsor (see Test Substance section).

2. The stability, uniformity of mixture and verification of concentration of alpha-Hexylcinnamaldehyde Technical (HCA) in its carriers during Product Safety Laboratories historical positive control study were not determined.

Study Director: _____
Daniel J. Merkel, B.S.
Product Safety Laboratories

4/20/06
Date

Submitter: _____
Signature

6/9/06
Date

Sponsor: _____
Signature

_____
Date

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QUALITY ASSURANCE STATEMENT

The Product Safety Laboratories' Quality Assurance Unit reviewed this study for adherence to PSL's Standard Operating Procedures, the study protocol, and all applicable GLP standards. This final report was found to be an accurate representation of the work conducted. Records of QA findings are kept on file. The summary below provides verification of statements made in the final report section that addresses Quality Assurance audits.

QA activities for this study:

QA Activity	Date Conducted	Date Findings Reported To Study Director And Management
Protocol review	6/7/05[1]; 8/10/05	6/7/05; 8/10/05
In-process inspection: *24 hour scoring (2^{nd} induction); Day 29 in-life food consumption observations*	6/17/05; 7/8/05	8/10/05
Raw data audit	8/10/05	8/10/05
Draft report review	8/10/05	8/10/05
Final report review		

Rhonda S. Krick, B.S.
Quality Assurance Director
Product Safety Laboratories

[1] PSL's "generic" protocol used for this study was reviewed by the Quality Assurance group on this date.

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DELAYED CONTACT DERMAL SENSITIZATION TEST - MODIFIED BUEHLER METHOD FOR SOLID MATERIALS

PROTOCOL NO.: P328.OEHHA

AGENCY: EPA (FIFRA)

STUDY NUMBER: 17608

SPONSOR: CALIFORNIA EPA,
OFFICE OF ENVIRONMENTAL HEALTH
HAZARD ASSESSMENT
1515 Clay Street, 16th Floor
Oakland, CA 94612

TEST SUBSTANCE IDENTIFICATION: Rubber Tiles, EPDM Tiles, High Density Polyethylene, and Rubber Crumb

PRODUCT IDENTIFICATION	PSL REFERENCE NUMBER
Rubber Tiles	041215-2D
EPDM Tiles	041215-3D
High Density Polyethylene	041215-4D
Rubber Crumb	050110-3D

TEST SUBSTANCE DESCRIPTION: Rubber tiles with cylindrical "feet", rust-colored tiles, a white sheet, and black rubber pieces

DATES RECEIVED: December 15, 2004 and January 10, 2005

STUDY INITIATION DATE: June 8, 2005

DATES OF TEST: June 9 - July 16, 2005

NOTEBOOK NO.: 05-49: pages 55-99

1. PURPOSE

To determine the potential for Rubber Tiles, EPDM Tiles, High Density Polyethylene, and Rubber Crumb to elicit a skin sensitization reaction.

2. SUMMARY

A dermal sensitization test was conducted with guinea pigs to determine the potential for Rubber Tiles, EPDM Tiles, High Density Polyethylene, and Rubber Crumb to produce sensitization after repeated topical applications.

The appropriate test substances were each topically applied to a group of ten healthy test guinea pigs, once each week for a three-week induction period. Twenty-eight days after the first induction dose, a challenge dose of the appropriate test substance was applied to a naive site on each guinea pig. Approximately 24 and 48 hours after each induction and challenge dose, the animals were scored for erythema.

A separate positive control study was conducted concurrently as PSL #17608. Due to the absence of a clear positive response at challenge, it was necessary to conduct a rechallenge in that study. Therefore, seven days after the primary challenge, a rechallenge was also conducted in this study using the original test animals. Approximately 24 and 48 hours after rechallenge patch removal the animals were scored for a sensitization response (erythema).

A table summarizing the incidence and severity of the sensitization response noted after challenge and rechallenge is found below:

Group 1

	Sensitization Response Indices			
	Incidence of Positive Response[1]		Severity[2]	
	Hours		Hours	
	24	48	24	48
Test Animals	0/10	0/10	0.10	0.0
Test Animals – Rechallenge	0/10	0/10	0.05	0.0

Group 2

	Sensitization Response Indices			
	Incidence of Positive Response[1]		Severity[2]	
	Hours		Hours	
	24	48	24	48
Test Animals	0/10	0/10	0.05	0.0
Test Animals – Rechallenge	0/10	0/10	0.10	0.0

[1] Animals with scores greater than 0.5.

[2] Sum of the erythema scores divided by the number of animals evaluated.

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Group 3

	Sensitization Response Indices			
	Incidence of Positive Response[1]		Severity[2]	
	Hours		Hours	
	24	48	24	48
Test Animals	0/10	0/10	0.10	0.0
Test Animals – Rechallenge	0/10	0/10	0.05	0.0

Group 4

	Sensitization Response Indices			
	Incidence of Positive Response[1]		Severity[2]	
	Hours		Hours	
	24	48	24	48
Test Animals	0/9[3]	0/9	0.17	0.0
Test Animals – Rechallenge	0/9	0/9	0.0	0.0

Group 5

	Sensitization Response Indices			
	Incidence of Positive Response[1]		Severity[2]	
	Hours		Hours	
	24	48	24	48
Test Animals	0/10	0/10	0.20	0.0
Test Animals – Rechallenge	0/10	0/10	0.05	0.0

Group 6

	Sensitization Response Indices			
	Incidence of Positive Response[1]		Severity[2]	
	Hours		Hours	
	24	48	24	48
Test Animals	0/10	0/10	0.10	0.0
Test Animals – Rechallenge	0/10	0/10	0.15	0.0

[1] Animals with scores greater than 0.5.

[2] Sum of the erythema scores divided by the number of animals evaluated.

[3] Animal #25768 was euthanized for humane reasons.

Group 7

	Sensitization Response Indices			
	Incidence of Positive Response[1]		Severity[2]	
	Hours		Hours	
	24	48	24	48
Test Animals	0/10	0/10	0.15	0.0
Test Animals – Rechallenge	0/10	0/10	0.10	0.0

Based on the results of this study, the test substances are not considered to be contact sensitizers. The positive response observed in the positive control validation study validates the test system used in this study (see Section 7).

3. MATERIALS

A. Test Substance

The test substances were identified as follows:

Material	**Rubber Tiles**	**EPDM Tiles**	**High Density Polyethylene**	**Rubber Crumb**
PSL Ref. #	041215-2D	041215-3D	041215-4D	050110-3D
Receipt Date	12/15/04	12/15/04	12/15/04	1/10/05
Composition	Shredded automobile tires held together with a polyurethane binder	Ethylene propylene diene monomer granules held together with a polyurethane binder	High density polyethylene	Shredded automobile tires
Description	Rubber tires with cylindrical "feet"	Rust-colored tiles	White sheet	Black rubber pieces
Documentation of the methods of synthesis, fabrication, or derivation of the test article is retained by:				
	Unity Surfacing Systems 56 Bloomingdale Rd. Hickesville, NY 11801	All About Play 3844 Presidio St. Sacramento, CA 95838	TOLAS Health Care Packaging 905 Pennsylvania Blvd. Feasterville, PA 19053	West Coast Rubber Recycling 105 Leavesly Rd., #7B Gilroy, CA 95020
The test substances were expected to be stable for the duration of testing.				

[1] Animals with scores greater than 0.5.

[2] Sum of the erythema scores divided by the number of animals evaluated.

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B. Animals

Ten test animals were indiscriminately assigned to each of the following test groups:

ANIMAL GROUP ASSIGNMENT

Group #	Induction Exposure	Challenge Exposure
1	High Density Polyethylene, 041215-4D (negative control)	High Density Polyethylene, 041215-4D (negative control)
2	High Density Polyethylene, 041215-4D (negative control)	Rubber Crumb, 050110-3D (test substance)
3	Rubber Crumb, 050110-3D (test substance)	Rubber Crumb, 050110-3D (test substance)
4	High Density Polyethylene, 041215-4D (negative control)	Rubber Tiles, 041215-2D (test substance)
5	Rubber Tiles, 041215-2D (test substance)	Rubber Tiles, 041215-2D (test substance)
6	High Density Polyethylene, 041215-4D (negative control)	EPDM Tiles, 041215-3D (test substance)
7	EPDM Tiles, 041215-3D (test substance)	EPDM Tiles, 041215-3D (test substance)

3.B.1 Number of Animals: 70

3.B.2 Sex: Male

3.B.3 Species/Strain: Guinea pigs/Hartley albino.

3.B.4 Age/Body weight: Test and Challenge/Rechallenge Groups: Young adult/males 319-418 grams at experimental start.

3.B.5 Source: Received from Elm Hill Breeding Labs, Chelmsford, MA on June 1, 2005 (Test Group).

4. METHODS

A. Husbandry

4.A.1 Housing: The animals were group housed in suspended stainless steel caging with mesh floors or plastic perforated bottom caging which conform to the size recommendations in the most recent *Guide for the Care and Use of Laboratory Animals DHEW (NIH)*. Litter paper was placed beneath the cage and was changed at least three times per week.

4.A.2 Animal Room Temperature Range: 18-23 °C

4.A.3 Photoperiod: 12-hour light/dark cycle

4.A.4 Acclimation Period: 8 days

4.A.5 Food: Pelleted Purina Guinea Pig Chow #5025

4.A.6 Water: Filtered tap water was supplied *ad-libitum* by an automatic water dispensing system.

4.A.7 Contaminants: There were no known contaminants reasonably expected to be found in the food or water at levels which would have interfered with the results of this study. Analyses of the food and water are conducted at least once a year and the records are kept on file at Product Safety Laboratories.

B. Identification

4.B.1 Cage: Each cage was identified with a cage card indicating at least the study number and identification and sex of the animal.

4.B.2 Animal: Each guinea pig was marked with a color code and given a sequential animal number assigned to study 17608, which constituted unique identification.

5. PROCEDURE

A. Preparation and Selection of Animals

On the day before initiation, the fur of a group of animals was removed by clipping the dorsal area and flanks. After clipping and prior to initiation, the animals were weighed and the skin was checked for any abnormalities. Only healthy animals without pre-existing skin irritation were selected for test. Animals were re-clipped prior to each dose.

B. Pre-Dose Scoring

Prior to each application, the dose sites were scored according to the scoring system described in Section 5.F.

C. Induction Phase

Once each week for three weeks, the appropriate test substance was moistened with distilled water (0.1 ml) and applied to the left side of each test animal using a 2 x 2-inch, 4-ply gauze patch. The sites were covered with dental dam and then wrapped with non-allergenic surgical tape to avoid dislocation of the patches and to minimize loss of the test substance. The High Density Polyethylene (Groups 1, 2, 4, and 6) and EPDM Tiles (Group 7) were applied as 1 x 1-inch squares. The Rubber Crumb (Group 3) and Rubber Tiles (Group 5) were applied using 0.4 g of Rubber Crumb and 1-inch diameter circles of the tiles, respectively. After the 6-hour exposure period, the patches were removed and the test sites were gently cleansed of any residual test substance. Approximately 24 and 48 hours after each induction application, readings were made of local reactions (erythema) according to the scoring system described in Section 5.F.

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D. Challenge Phase

Twenty-eight days after the first induction dose, the appropriate test substance was moistened with distilled water (0.1 ml) and applied to the left side of each test animal using a 2 x 2-inch, 4-ply gauze patch to a naive site on the right side of each animal as a challenge dose, using the procedures described above. These sites were evaluated for a sensitization response (erythema) approximately 24 and 48 hours after the challenge application according to the system described in Section 5.F.

E. Rechallenge Phase

Due to the absence of a positive response in animals at the challenge dose in the positive control study, the Study Director, in consultation with the Sponsor, elected to conduct a rechallenge. Seven days after the primary challenge, a rechallenge was conducted using the original test animals. Approximately 24 and 48 hours after rechallenge patch removal, the animals were scored for a sensitization response (erythema). The test substance, as received, was applied to a naive site on each test animal, using the procedures described in 5.C. Approximately 24 and 48 hours after rechallenge patch removal the animals were scored for a sensitization response (erythema). These sites were evaluated for a sensitization response (erythema) approximately 24 and 48 hours after the rechallenge application according to the system described in Section 5.F.

F. Scoring System

0 - no reaction
0.5 - very faint erythema, usually non-confluent*
1 - faint erythema, usually confluent
2 - moderate erythema
3 - severe erythema with or without edema

*Very faint erythema is not considered a positive reaction.

G. Body Weights

Individual body weights of the animals were recorded prior to initiation and again on the day after challenge.

H. Clinical Observations and Food Consumption

Clinical observations were made daily on all animals during the study. Food consumption was qualitatively evaluated daily, during clinical observations.

The results for the observations for all animals #'s 25730-25799 are as follows:

Day	Observations	
0	Clinical Observations	Food Consumption
1, 3-38	Active and healthy	Normal
2	Animal #25768 – Gasping, moribund, ano-genital staining, death imminent, euthanized for humane reasons. All other were active and healthy.	Normal

6. EVALUATION

In order to evaluate the sensitization response at challenge, two indices were used: one for incidence and one for severity (Ritz, H. and Buehler, E., 1980) in the test and vehicle control animals.

The incidence index is the ratio of animals with erythema scores greater than 0.5 per number of animals evaluated, and is presented for both the 24 and 48-hour intervals after challenge evaluation as follows:

Incidence Index = Number of erythema scores greater than 0.5 / Number of animals evaluated

The severity index is the mean erythema score, and is calculated for both the 24 and 48-hour intervals after challenge evaluation according to the following formula:

$$\text{Severity Index} = \frac{\text{Sum of erythema scores}}{\text{Number of animals evaluated}}$$

The following criteria were used to classify the test substance as a potential contact sensitizer (Robinson, et al., 1990):

At the 24-hour and/or 48-hour scoring interval, 15% or more of the test animals exhibit a positive response (scores > 0.5) in the absence of similar results in the vehicle control group.

The positive reaction at the 24-hour interval must persist to 48 hours in at least one test animal.

7. POSITIVE CONTROL VALIDATION STUDY

The procedures used in this study were validated using alpha-Hexylcinnamaldehyde Technical, 85% (HCA) as a positive control substance. The validation study, PSL Study #17609, was performed concurrent with this study by Product Safety Laboratories and testing was completed on July 16, 2005. The raw data and report for this study are archived in Product Safety Laboratories Data Notebook No. 05-49: pages 100-113. This test was conducted at the Dayton Facility with Hartley strain albino guinea pigs from Elm Hill Breeding Labs following induction and challenge procedures similar to those described in Section 5. The results obtained from this testing are presented in Section 13.

8. STUDY CONDUCT

This study was conducted at Product Safety Laboratories, 2394 Highway 130, Dayton, New Jersey 08810. The primary scientist for this study was Anselmo Villagran, B.S. This study was conducted to comply with the Good Laboratory Practice (GLP) regulations as defined in:

- 40 CFR 160: U.S. EPA GLP Standards: Pesticide Programs (FIFRA)

and in accordance with:

- U.S. EPA Health Effects Test Guidelines, OPPTS 870.2600 (2003)

9. REFERENCES

Robinson, M., Nusair, T., Fletcher, E., and Ritz, H., A Review of the Buehler Guinea Pig Skin Sensitization Test And Its Use in a Risk Assessment Process for Human Skin Sensitization. *Toxicology*, 61, 91-107, 1990.

Ritz, H., and Buehler, E., Planning, Conduct, and Interpretation Of Guinea Pig Sensitization Patch Tests. *Current Concepts in Cutaneous Toxicity*, V.A. Drill and P. Lazar (Eds.), Academic Press, New York, 1980, page 25.

10. QUALITY ASSURANCE

The final report was audited for agreement with the raw data records and for compliance with the protocol, Product Safety Laboratories Standard Operating Procedures and appropriate Good Laboratory Practice Standards. Dates of inspections and audits performed during the study, and the dates of reporting of the inspection and audit findings to the Study Director and Facility Management are presented in the Quality Assurance Statement.

11. DEVIATIONS FROM FINAL PROTOCOL

None.

12. FINAL REPORT AND RECORDS TO BE MAINTAINED

The original, signed final report will be forwarded to the Sponsor. A copy of this signed report, together with the protocol and all raw data generated at Product Safety Laboratories, is maintained in the Product Safety Laboratories Archives. PSL will maintain these records for a period of at least five years. After this time, the Sponsor will be offered the opportunity to take possession of the records or will be charged an archiving fee for continued archiving by PSL.

13. RESULTS

Individual body weights for test and positive control animals are presented in Tables 1 and 2. Pre-Dose, Induction, Challenge, and Rechallenge Phase skin reaction scores for test and positive control animals are presented in Tables 3 through 8.

Due to the fact that death appeared imminent, one test animal was euthanized for humane reasons within the initial 48 hours during the first induction. Toxic signs noted prior to death included gasping, hypoactivity, ano-genital staining, and moribund appearance. Gross necropsy of the decedent revealed discoloration of the lungs, intestines, and entire area of the reproductive organs. However, all other animals appeared active and healthy during the entire observation period.

Induction Phase

Test Animal Groups 1, 2, 4, and 6 - (100% High Density Polyethylene, as received): Very faint erythema (0.5) was noted for some test sites during the induction phase.

Test Animal Group 3 - (100% Rubber Crumb, as received): Very faint erythema (0.5) was noted for some test sites during the induction phase.

Test Animal Group 5 - (100% Rubber Tiles, as received): Very faint erythema (0.5) was noted for some test sites during the induction phase.

Test Animal Group 7 - (100% EPDM Tiles, as received): Very faint erythema (0.5) was noted for some test sites during the induction phase.

Positive Control Animals (HCA applied undiluted): Very faint erythema (0.5) was noted for most test sites during the induction phase.

Challenge Phase

Test Animal Group 1 - (100% High Density Polyethylene): Very faint erythema (0.5) was noted for two of ten test sites 24 hours following the challenge application. Irritation cleared from all sites by 48 hours.

Test Animal Group 2 - (100% Rubber Crumb): Very faint erythema (0.5) was noted for one of ten test sites 24 hours following the challenge application. Irritation cleared from all sites by 48 hours.

Test Animal Group 3 - (100% Rubber Crumb): Very faint erythema (0.5) was noted for two of ten test sites 24 hours following the challenge application. Irritation cleared from all sites by 48 hours.

Test Animal Group 4 - (100% Rubber Tiles): Very faint erythema (0.5) was noted for three of nine test sites 24 hours following the challenge application. Irritation cleared from all sites by 48 hours.

Test Animal Group 5 - Test Animals (100% Rubber Tiles): Very faint erythema (0.5) was noted for four of ten test sites 24 hours following the challenge application. Irritation cleared from all sites by 48 hours.

Test Animal Group 6 - Test Animals (100% EPDM Tiles): Very faint erythema (0.5) was noted for two of ten test sites 24 hours following the challenge application. Irritation cleared from all sites by 48 hours.

Test Animal Group 7- Test Animals (100% EPDM Tiles): Very faint erythema (0.5) was noted for three of nine test sites 24 hours following the challenge application. Irritation cleared from all sites by 48 hours.

Positive Control Animals (HCA applied undiluted): Very faint erythema (0.5) was noted for three of ten test sites 24 hours following the challenge application. Irritation cleared from all sites by 48 hours.

Naive Control Animals (HCA applied undiluted): Very faint erythema (0.5) was noted for one of five naive control sites 24 hours after challenge. Irritation cleared from all sites by 48 hours.

Rechallenge Phase

Test Animal Group 1 - (100% High Density Polyethylene): Very faint erythema (0.5) was noted for one of ten test sites 24 hours following the rechallenge application. Irritation cleared from all sites by 48 hours.

Test Animal Group 2 - (100% Rubber Crumb): Very faint erythema (0.5) was noted for two of ten test sites 24 hours following the rechallenge application. Irritation cleared from all sites by 48 hours.

Test Animal Group 3 - (100% Rubber Crumb): Very faint erythema (0.5) was noted for one of ten test sites 24 hours following the rechallenge application. Irritation cleared from all sites by 48 hours.

Test Animal Group 4 - (100% Rubber Tiles): Very faint erythema (0.5) was noted for two of nine test sites 24 hours following the rechallenge application. Irritation cleared from all sites by 48 hours.

Test Animal Group 5 - Test Animals (100% Rubber Tiles): Very faint erythema (0.5) was noted for one of ten test sites 24 hours following the rechallenge application. Irritation cleared from all sites by 48 hours.

Test Animal Group 6 - Test Animals (100% EPDM Tiles): Very faint erythema (0.5) was noted for three of ten test sites 24 hours following the rechallenge application. Irritation cleared from all sites by 48 hours.

Test Animal Group 7- Test Animals (100% EPDM Tiles): Very faint erythema (0.5) was noted for two of ten test sites 24 hours following the rechallenge application. Irritation cleared from all sites by 48 hours.

Positive Control Animals (HCA applied undiluted): Five of ten animals exhibited signs of a sensitization response [faint to severe erythema (1-3)] 24 hours after rechallenge. Similar irritation persisted for four sites through 48 hours. Very faint erythema (0.5) was observed at two test sites.

Naive Control Animals (HCA applied undiluted): Very faint erythema (0.5) was noted at two of five naive control sites 24 hours after challenge. Irritation cleared from all sites by 48 hours.

14. CONCLUSION

Based on these findings and on the evaluation system used, Rubber Tiles, EPDM Tiles, High Density Polyethylene, and Rubber Crumb are not considered to be contact sensitizers.

The positive response observed in the positive control validation study with alpha-Hexylcinnamaldehyde Technical (HCA) validates the test system used in this study (see Section 7).

SIGNATURE

Rubber Tiles, EPDM Tiles, High Density Polyethylene, and Rubber Crumb

I, the undersigned, declare that the methods, results and data contained in this report faithfully reflect the procedures used and raw data collected during the study.

Daniel J. Merkel, B.S.
Study Director
Product Safety Laboratories

Date

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TABLE 1: INDIVIDUAL BODY WEIGHTS (TEST SUBSTANCE)

Test Substance

Group 1

Animal No.	Sex	Initial (g)	Day After Challenge (g)
25730	M	383	596
25731	M	385	550
25732	M	389	629
25733	M	387	617
25734	M	374	565
25735	M	387	564
25736	M	355	566
25737	M	361	574
25738	M	347	601
25739	M	406	633

Group 2

Animal No.	Sex	Initial (g)	Day After Challenge (g)
25740	M	369	622
25741	M	360	618
25742	M	368	572
25743	M	363	515
25744	M	364	571
25745	M	351	568
25746	M	355	556
25747	M	351	563
25748	M	373	608
25749	M	414	625

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TABLE 1 (cont.): INDIVIDUAL BODY WEIGHTS (TEST SUBSTANCE)

Test Substance

Group 3

Animal No.	Sex	Initial (g)	Day After Challenge (g)
25750	M	365	561
25751	M	412	696
25752	M	375	583
25753	M	372	639
25754	M	381	545
25755	M	375	527
25756	M	366	526
25757	M	369	487
25758	M	357	508
25759	M	369	575

Group 4

Animal No.	Sex	Initial (g)	Day After Challenge (g)
25760	M	377	551
25761	M	362	548
25762	M	371	684
25763	M	417	565
25764	M	375	611
25765	M	355	533
25766	M	376	558
25767	M	399	584
25768	M	319	-[1]
25769	M	366	489

[1] Animal #25768 was euthanized for humane reasons, body weight was taken prior to euthanasia (315 g).

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TABLE 1 (cont.): INDIVIDUAL BODY WEIGHTS (TEST SUBSTANCE)

Test Substance

Group 5

Animal No.	Sex	Initial (g)	Day After Challenge (g)
25770	M	361	633
25771	M	368	607
25772	M	378	633
25773	M	375	686
25774	M	370	591
25775	M	379	664
25776	M	384	674
25777	M	354	623
25778	M	346	562
25779	M	386	655

Group 6

Animal No.	Sex	Initial (g)	Day After Challenge (g)
25780	M	418	606
25781	M	361	548
25782	M	372	571
25783	M	381	615
25784	M	363	491
25785	M	370	520
25786	M	371	597
25787	M	382	652
25788	M	365	598
25789	M	382	648

TABLE 1 (cont.): INDIVIDUAL BODY WEIGHTS (TEST SUBSTANCE)

Test Substance

Group 7

Animal No.	Sex	Initial (g)	Day After Challenge (g)
25790	M	374	574
25791	M	358	589
25792	M	371	615
25793	M	409	707
25794	M	385	557
25795	M	387	545
25796	M	368	553
25797	M	391	597
25798	M	368	581
25799	M	385	575

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TABLE 2: INDIVIDUAL BODY WEIGHTS (POSITIVE CONTROL)

Positive Control Validation Study[1]

Positive Control Group

Animal No.	Sex	Initial (g)	Day After Challenge (g)
25800	M	426	656
25801	M	373	658
25802	M	356	679
25803	M	367	621
25804	M	404	684
25805	M	381	612
25806	M	389	605
25807	M	399	657
25808	M	387	668
25809	M	381	560

Naive Control Group

Animal No.	Sex	Initial (g)	Day After Challenge (g)
25810	M	357	614
25811	M	384	631
25812	M	347	573
25813	M	383	661
25814	M	358	530

[1] PSL Study #17609, performed by PSL, concurrent with this study. Testing was completed on July 16, 2005.

PSL PRODUCT SAFETY LABORATORIES

TABLE 3: PRE-DOSE AND INDUCTION PHASE SKIN REACTION SCORES (TEST SUBSTANCE)

Test Substance

Group 1

PRE-DOSE

Induction Number	1	2	3
Concentration	100%	100%	100%
Amount Applied[1]	N/A	N/A	N/A
Hours[2]	Pre-Dose Scoring		
Animal No.			
25730	0	0	0
25731	0	0	0
25732	0	0	0
25733	0	0	0
25734	0	0	0
25735	0	0	0
25736	0	0	0
25737	0	0	0
25738	0	0	0
25739	0	0	0

INDUCTION

Induction Number	1		2		3	
Concentration	100%		100%		100%	
Amount Applied[1]	N/A		N/A		N/A	
Hours[2]	24	48	24	48	24	48
Animal No.						
25730	0	0	0	0	0	0
25731	0	0	0	0	0	0
25732	0	0	0.5	0	0	0
25733	0.5	0	0	0	0	0
25734	0	0	0	0	0	0
25735	0	0	0.5	0	0	0
25736	0	0	0	0	0	0
25737	0	0	0	0	0	0
25738	0	0	0	0	0	0
25739	0	0	0.5	0	0	0

[1] The test substance was moistened with distilled water and applied as 1 x 1-inch squares.

[2] Hours after induction dose.

PRODUCT SAFETY LABORATORIES

TABLE 3 (cont.): PRE-DOSE AND INDUCTION PHASE SKIN REACTION SCORES (TEST SUBSTANCE)

Test Substance

Group 2

PRE-DOSE

Induction Number	1	2	3
Concentration	100%	100%	100%
Amount Applied[1]	N/A	N/A	N/A
Hours[2]	Pre-Dose Scoring		
Animal No.			
25740	0	0	0
25741	0	0	0
25742	0	0	0
25743	0	0	0
25744	0	0	0
25745	0	0	0
25746	0	0	0
25747	0	0	0
25748	0	0	0
25749	0	0	0

INDUCTION

Induction Number	1		2		3	
Concentration	100%		100%		100%	
Amount Applied[1]	N/A		N/A		N/A	
Hours[2]	24	48	24	48	24	48
Animal No.						
25740	0	0	0	0	0	0
25741	0	0	0.5	0	0	0
25742	0	0	0.5	0.5	0.5	0
25743	0	0	0	0	0	0
25744	0	0	0	0	0	0
25745	0	0	0	0	0	0
25746	0	0	0	0	0	0
25747	0	0	0	0	0	0
25748	0	0	0	0	0	0
25749	0	0	0	0	0	0

[1] The test substance was moistened with distilled water and applied as 1 x 1-inch squares.

[2] Hours after induction dose.

PSL PRODUCT SAFETY LABORATORIES

TABLE 3 (cont.): PRE-DOSE AND INDUCTION PHASE SKIN REACTION SCORES (TEST SUBSTANCE)

Test Substance

Group 3

PRE-DOSE

Induction Number	1	2	3
Concentration[1]	100%	100%	100%
Amount Applied (g)	0.4	0.4	0.4
Hours[2]	Pre-Dose Scoring		
Animal No.			
25750	0	0	0
25751	0	0	0
25752	0	0	0
25753	0	0	0
25754	0	0	0
25755	0	0	0
25756	0	0	0
25757	0	0	0
25758	0	0	0
25759	0	0	0

INDUCTION

Induction Number	1		2		3	
Concentration[1]	100%		100%		100%	
Amount Applied (g)	0.4		0.4		0.4	
Hours[2]	24	48	24	48	24	48
Animal No.						
25750	0.5	0.5	0	0	0	0
25751	0	0	0	0	0	0
25752	0	0	0.5	0	0	0
25753	0	0	0	0	0.5	0
25754	0.5	0	0	0	0	0
25755	0	0	0	0	0	0
25756	0	0	0.5	0	0	0
25757	0	0	0	0	0	0
25758	0	0	0	0	0	0
25759	0	0	0	0	0	0

[1] The test substance was moistened with distilled water and applied

[2] Hours after induction dose.

PSL PRODUCT SAFETY LABORATORIES

TABLE 3 (cont.): PRE-DOSE AND INDUCTION PHASE SKIN REACTION SCORES (TEST SUBSTANCE)

Test Substance

Group 4

PRE-DOSE

Induction Number	1	2	3
Concentration[1]	100%	100%	100%
Amount Applied	N/A	N/A	N/A
Hours[2]	Pre-Dose Scoring		
Animal No.			
25760	0	0	0
25761	0	0	0
25762	0	0	0
25763	0	0	0
25764	0	0	0
25765	0	0	0
25766	0	0	0
25767	0	0	0
25768	0	-[3]	-
25769	0	0	0

INDUCTION

Induction Number	1		2		3	
Concentration[1]	100%		100%		100%	
Amount Applied	N/A		N/A		N/A	
Hours[2]	24	48	24	48	24	48
Animal No.						
25760	0.5	0.5	0	0	0	0
25761	0	0	0	0	0	0
25762	0	0	0	0	0	0
25763	0	0	0.5	0	0	0
25764	0	0	0	0	0	0
25765	0	0	0	0	0	0
25766	0	0	0.5	0	0	0
25767	0	0	0	0	0	0
25768	0	-[3]	-	-	-	-
25769	0	0	0.5	0	0	0

[1] The test substance was moistened with distilled water and applied as 1 x 1-inch squares.

[2] Hours after induction dose.

[3] Animal #25768 was euthanized for humane reasons.

PRODUCT SAFETY LABORATORIES

TABLE 3 (cont.): PRE-DOSE AND INDUCTION PHASE SKIN REACTION SCORES (TEST SUBSTANCE)

Test Substance

Group 5

PRE-DOSE

Induction Number	1	2	3
Concentration[1]	100%	100%	100%
Amount Applied	N/A	N/A	N/A
Hours[2]	Pre-Dose Scoring		
Animal No.			
25770	0	0	0
25771	0	0	0
25772	0	0	0
25773	0	0	0
25774	0	0	0
25775	0	0	0
25776	0	0	0
25777	0	0	0
25778	0	0	0
25779	0	0	0

INDUCTION

Induction Number	1		2		3	
Concentration[1]	100%		100%		100%	
Amount Applied	N/A		N/A		N/A	
Hours[2]	24	48	24	48	24	48
Animal No.						
25770	0.5	0	0	0	0	0
25771	0	0	0	0	0	0
25772	0.5	0.5	0.5	0	0	0
25773	0	0	0	0	0	0
25774	0	0	0	0	0	0
25775	0	0	0	0	0	0
25776	0.5	0.5	0.5	0	0	0
25777	0	0	0	0	0	0
25778	0.5	0.5	0.5	0	0	0
25779	0	0	0.5	0	0	0

[1] The test substance was moistened with distilled water and applied as one-inch diameter circles.

[2] Hours after induction dose.

PRODUCT SAFETY LABORATORIES

TABLE 3 (cont.): PRE-DOSE AND INDUCTION PHASE SKIN REACTION SCORES (TEST SUBSTANCE)

Test Substance

Group 6

PRE-DOSE

Induction Number	1	2	3
Concentration[1]	100%	100%	100%
Amount Applied	N/A	N/A	N/A
Hours[2]	Pre-Dose Scoring		
Animal No.			
25780	0	0	0
25781	0	0	0
25782	0	0	0
25783	0	0	0
25784	0	0	0
25785	0	0	0
25786	0	0	0
25787	0	0	0
25788	0	0	0
25789	0	0	0

INDUCTION

Induction Number	1		2		3	
Concentration[1]	100%		100%		100%	
Amount Applied	N/A		N/A		N/A	
Hours[2]	24	48	24	48	24	48
Animal No.						
25780	0	0	0	0	0	0
25781	0	0	0.5	0	0	0
25782	0	0	0.5	0	0	0
25783	0	0	0	0	0	0
25784	0	0	0	0	0	0
25785	0	0	0	0	0.5	0
25786	0.5	0.5	0	0	0	0
25787	0	0	0	0	0	0
25788	0	0	0	0	0	0
25789	0	0	0	0	0	0

[1] The test substance was moistened with distilled water and applied as 1 x 1-inch squares.

[2] Hours after induction dose.

PSL PRODUCT SAFETY LABORATORIES

TABLE 3 (cont.): PRE-DOSE AND INDUCTION PHASE SKIN REACTION SCORES (TEST SUBSTANCE)

Test Substance

Group 7

PRE-DOSE

Induction Number	1	2	3
Concentration[1]	100%	100%	100%
Amount Applied	N/A	N/A	N/A
Hours[2]	Pre-Dose Scoring		
Animal No.			
25790	0	0	0
25791	0	0	0
25792	0	0	0
25793	0	0	0
25794	0	0	0
25795	0	0	0
25796	0	0	0
25797	0	0	0
25798	0	0	0
25799	0	0	0

INDUCTION

Induction Number	1		2		3	
Concentration[1]	100%		100%		100%	
Amount Applied	N/A		N/A		N/A	
Hours[2]	24	48	24	48	24	48
Animal No.						
25790	0	0	0.5	0	0.5	0
25791	0	0	0	0	0	0
25792	0.5	0	0.5	0	0	0
25793	0	0	0	0	0	0
25794	0	0	0	0	0	0
25795	0	0	0.5	0	0	0
25796	0.5	0	0	0	0	0
25797	0	0	0	0	0	0
25798	0	0	0	0	0	0
25799	0	0	0	0	0.5	0

[1] The test substance was moistened with distilled water and applied as 1 x 1-inch squares.

[2] Hours after induction dose.

TABLE 4: PRE-DOSE AND INDUCTION PHASE SKIN REACTION SCORES (POSITIVE CONTROL)

Positive Control Validation Study[1]

Positive Control Group

PRE-DOSE

Induction Number	1	2	3
Concentration	Undiluted	Undiluted	Undiluted
Amount Applied (ml)	0.4	0.4	0.4
Hours[2]	Pre-Dose Scoring		
Animal No.			
25800	0	0	0
25801	0	0	0
25802	0	0	0
25803	0	0	0
25804	0	0	0
25805	0	0	0
25806	0	0	0
25807	0	0	0
25808	0	0	0
25809	0	0	0

INDUCTION

Induction Number	1		2		3	
Concentration	Undiluted		Undiluted		Undiluted	
Amount Applied (ml)	0.4		0.4		0.4	
Hours[2]	24	48	24	48	24	48
Animal No.						
25800	0	0	0	0	0.5	0.5
25801	0	0	0	0	0	0
25802	0.5	0	0.5	0	0	0
25803	0	0	0.5	0	0.5	0
25804	0	0	0	0	0	0
25805	0.5	0	0.5	0	0.5	0.5
25806	0	0	0	0	0	0
25807	0	0	0	0	0.5	0.5
25808	0	0	0.5	0	0.5	0
25809	0	0	0	0	0	0

[1] PSL Study #17609, performed by PSL, concurrent with this study. Testing was completed on July 16, 2005.

[2] Hours after induction dose.

TABLE 5: PRE-DOSE AND CHALLENGE PHASE SKIN REACTION SCORES (TEST SUBSTANCE)

Test Substance

Group 1[1]

Animal No.	Pre-Dose	Hours after Dosing	
		24	48
25730	0	0	0
25731	0	0	0
25732	0	0.5	0
25733	0	0	0
25734	0	0	0
25735	0	0	0
25736	0	0	0
25737	0	0	0
25738	0	0.5	0
25739	0	0	0

Group 2[2]

Animal No.	Pre-Dose	Hours after Dosing	
		24	48
25740	0	0	0
25741	0	0	0
25742	0	0	0
25743	0	0	0
25744	0	0	0
25745	0	0.5	0
25746	0	0	0
25747	0	0	0
25748	0	0	0
25749	0	0	0

[1] The test substance was moistened with distilled water and applied as 1 x 1-inch squares.

[2] The test substance was moistened with distilled water and applied.

PSL PRODUCT SAFETY LABORATORIES

TABLE 5 (cont.): PRE-DOSE AND CHALLENGE PHASE SKIN REACTION SCORES (TEST SUBSTANCE)

Test Substance

Group 3[1]

Animal No.	Pre-Dose	Hours after Dosing	
		24	48
25750	0	0	0
25751	0	0.5	0
25752	0	0	0
25753	0	0.5	0
25754	0	0	0
25755	0	0	0
25756	0	0	0
25757	0	0	0
25758	0	0	0
25759	0	0	0

Group 4[2]

Animal No.	Pre-Dose	Hours after Dosing	
		24	48
25760	0	0	0
25761	0	0	0
25762	0	0	0
25763	0	0	0
25764	0	0	0
25765	0	0.5	0
25766	0	0.5	0
25767	0	0.5	0
25768[3]	0	-	-
25769	0	0	0

[1] The test substance was moistened with distilled water and applied.

[2] The test substance was moistened with distilled water and applied as one-inch diameter circles.

[3] Animal #25768 was euthanized for humane reasons.

PSL PRODUCT SAFETY LABORATORIES

TABLE 5 (cont.): PRE-DOSE AND CHALLENGE PHASE SKIN REACTION SCORES (TEST SUBSTANCE)

Test Substance Group

Group 5[1]

Animal No.	Pre-Dose	Hours after Dosing	
		24	48
25770	0	0.5	0
25771	0	0	0
25772	0	0	0
25773	0	0.5	0
25774	0	0	0
25775	0	0.5	0
25776	0	0	0
25777	0	0	0
25778	0	0	0
25779	0	0.5	0

Group 6[2]

Animal No.	Pre-Dose	Hours after Dosing	
		24	48
25780	0	0.5	0
25781	0	0	0
25782	0	0	0
25783	0	0	0
25784	0	0	0
25785	0	0	0
25786	0	0	0
25787	0	0	0
25788	0	0.5	0
25789	0	0	0

[1] The test substance was moistened with distilled water and applied as one-inch diameter circles.

[2] The test substance was moistened with distilled water and applied as 1 x 1-inch squares.

PSL PRODUCT SAFETY LABORATORIES

TABLE 5 (cont.): PRE-DOSE AND CHALLENGE PHASE SKIN REACTION SCORES (TEST SUBSTANCE)

Test Substance Group[1]

Group 7

Animal No.	Pre-Dose	Hours after Dosing	
		24	48
25790	0	0	0
25791	0	0	0
25792	0	0.5	0
25793	0	0	0
25794	0	0.5	0
25795	0	0	0
25796	0	0	0
25797	0	0	0
25798	0	0.5	0
25799	0	0	0

[1] The test substance was moistened with distilled water and applied as 1 x 1-inch squares.

TABLE 6: PRE-DOSE AND CHALLENGE PHASE SKIN REACTION SCORES (POSITIVE CONTROL)

Positive Control Validation Study[1]

Positive Control Group[2]

Animal No.	Pre-Dose	Hours after Dosing	
		24	48
25800	0	0	0
25801	0	0	0
25802	0	0.5	0
25803	0	0	0
25804	0	0	0
25805	0	0	0
25806	0	0.5	0
25807	0	0	0
25808	0	0.5	0
25809	0	0	0

Naive Control Group[2]

Animal No.	Pre-Dose	Hours after Dosing	
		24	48
25810	0	0	0
25811	0	0	0
25812	0	0.5	0
25813	0	0	0
25814	0	0	0

[1] PSL Study #17609, performed by PSL, concurrent with this study. Testing was completed on July 16, 2005.

[2] Four-tenths of a milliliter of the test substance was applied undiluted.

PSL PRODUCT SAFETY LABORATORIES

TABLE 7: PRE-DOSE AND RECHALLENGE PHASE SKIN REACTION SCORES (TEST SUBSTANCE)

Test Substance

Group 1[1]

Animal No.	Pre-Dose	Hours after Dosing	
		24	48
25730	0	0	0
25731	0	0	0
25732	0	0	0
25733	0	0	0
25734	0	0	0
25735	0	0	0
25736	0	0	0
25737	0	0	0
25738	0	0.5	0
25739	0	0	0

Group 2[2]

Animal No.	Pre-Dose	Hours after Dosing	
		24	48
25740	0	0.5	0
25741	0	0	0
25742	0	0	0
25743	0	0	0
25744	0	0	0
25745	0	0	0
25746	0	0.5	0
25747	0	0	0
25748	0	0	0
25749	0	0	0

[1] The test substance was moistened with distilled water and applied as 1 x 1-inch squares.

[2] The test substance was moistened with distilled water and applied.

PSL PRODUCT SAFETY LABORATORIES

TABLE 7 (cont.): PRE-DOSE AND RECHALLENGE PHASE SKIN REACTION SCORES (TEST SUBSTANCE)

Test Substance

Group 3[1]

Animal No.	Pre-Dose	Hours after Dosing	
		24	48
25750	0	0	0
25751	0	0	0
25752	0	0	0
25753	0	0	0
25754	0	0	0
25755	0	0	0
25756	0	0	0
25757	0	0.5	0
25758	0	0	0
25759	0	0	0

Group 4[2]

Animal No.	Pre-Dose	Hours after Dosing	
		24	48
25760	0	0	0
25761	0	0	0
25762	0	0	0
25763	0	0	0
25764	0	0	0
25765	0	0	0
25766	0	0	0
25767	0	0	0
25768[3]	0	-	-
25769	0	0	0

[1] The test substance was moistened with distilled water and applied.

[2] The test substance was moistened with distilled water and applied as one-inch diameter circles.

[3] Animal #25768 was euthanized for humane reasons.

PSL PRODUCT SAFETY LABORATORIES

TABLE 7 (cont.): PRE-DOSE AND RECHALLENGE PHASE SKIN REACTION SCORES (TEST SUBSTANCE)

Test Substance

Group 5[1]

Animal No.	Pre-Dose	Hours after Dosing	
		24	48
25770	0	0	0
25771	0	0	0
25772	0	0	0
25773	0	0	0
25774	0	0	0
25775	0	0	0
25776	0	0.5	0
25777	0	0	0
25778	0	0	0
25779	0	0	0

Group 6[2]

Animal No.	Pre-Dose	Hours after Dosing	
		24	48
25780	0	0	0
25781	0	0	0
25782	0	0	0
25783	0	0.5	0
25784	0	0	0
25785	0	0.5	0
25786	0	0	0
25787	0	0	0
25788	0	0.5	0
25789	0	0	0

[1] The test substance was moistened with distilled water and applied as one-inch diameter circles.

[2] The test substance was moistened with distilled water and applied as 1 x 1-inch squares.

PSL PRODUCT SAFETY LABORATORIES

TABLE 7 (cont.): PRE-DOSE AND RECHALLENGE PHASE SKIN REACTION SCORES (TEST SUBSTANCE)

Test Substance

Group 7[1]

Animal No.	Pre-Dose	Hours after Dosing	
		24	48
25790	0	0	0
25791	0	0	0
25792	0	0	0
25793	0	0	0
25794	0	0.5	0
25795	0	0	0
25796	0	0	0
25797	0	0.5	0
25798	0	0	0
25799	0	0	0

[1] The test substance was moistened with distilled water and applied as 1 x 1-inch squares.

PSL PRODUCT SAFETY LABORATORIES

TABLE 8: PRE-DOSE AND RECHALLENGE PHASE SKIN REACTION SCORES (POSITIVE CONTROL)

Positive Control Validation Study[1]

Positive Control Group[2]

Animal No.	Pre-Dose	Hours after Dosing	
		24	48
25800	0	1	0.5
25801	0	0.5	0
25802	0	2	1
25803	0	0.5	0
25804	0	3	2
25805	0	2	2
25806	0	0	0
25807	0	3	3
25808	0	0	0
25809	0	0	0

Naive Control Group[2]

Animal No.	Pre-Dose	Hours after Dosing	
		24	48
26548	0	0	0
26549	0	0.5	0
26550	0	0.5	0
26551	0	0	0
26552	0	0	0

[1] PSL Study #17609, performed by PSL, concurrent with this study. Testing was completed on July 16, 2005.

[2] Four-tenths of a milliliter of the test substance was applied undiluted.